

Antimicrobial Potential and Resistance of Savannah Soil Microbiomes Near *Juniperus ashei* and *Opuntia engelmannii* at the Lady Bird Johnson Wildflower Center

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Introduction

Soil microbiomes are a fundamental yet often overlooked part of One Health, containing some of the most diverse microbial communities on Earth. These microbial communities drive nutrient cycling, ecosystem resilience, and the production of antimicrobial compounds that are critical to antibiotic discovery (Bahram et al.). The global rise of antibiotic resistance has created a need for antimicrobial compounds, and soil bacteria are one of the most promising yet underexplored sources. While general patterns of soil microbiome diversity have been researched, the majority of soil bacteria remain unstudied. This means that the majority of antibiotic-producing organisms have yet to be studied (Hutchings et al., 2019). Tiny Earth is an undergraduate research experience that addresses the crises of antibiotic resistance and declining soil health by immersing students in their own research, consisting of collecting soil, isolating bacterial colonies, and looking for potential antimicrobial activity (Tiny Earth). Through Tiny Earth, soil was collected from the Lady Bird Johnson Wildflower Center, a native plant conservation site that regularly uses controlled burns to restore ecosystem health (LBJ Wildflower Center). The sample was collected from an open Savannah near a couple of plant species, specifically *Juniperus ashei* and *Opuntia engelmannii*. *Juniperus* species contain essential oils that exhibit antibacterial properties, specifically against Gram-positive bacteria and pathogens. These characteristics can disrupt nearby microbial activity, potentially favoring antibiotic resistant species (Spengler et al.).

Research Question

How do different bacterial colonies collected from soil near *Juniperus ashei* and *Opuntia engelmannii* impact the Gram classification, morphological characteristics, and antibiotic resistance profiles of isolated bacteria?



Figure 1. Two images showing the *Juniperus ashei* and *Opuntia engelmannii* near the soil collection site.

Methods

Soil Collection and Serial Dilution



Figure 2. Collecting soil for testing



Figure 3. Soil dilutions 10⁻¹ on far left

Plating Dilutions and Choosing 32 Colonies for Master Plates



Figure 4. Side A 10⁻¹ dilution plate



Figure 10. Soil sample after collection

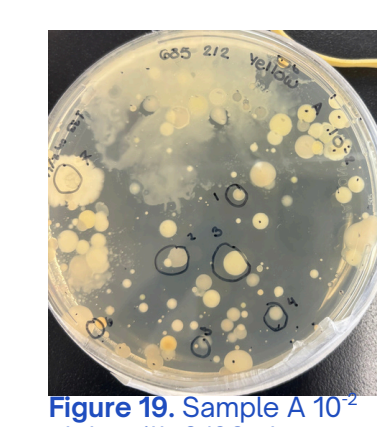


Figure 19. Sample A 10⁻¹ plate with 8/32 chosen colonies circled

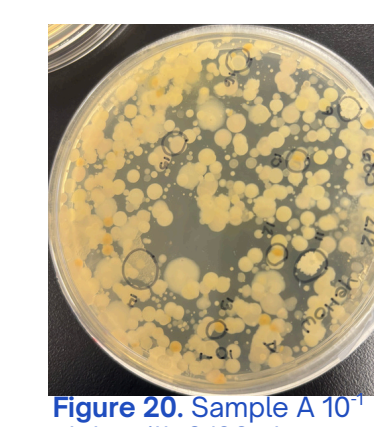


Figure 20. Sample A 10⁻² plate with 8/32 chosen colonies circled

Colony Morphology Observation

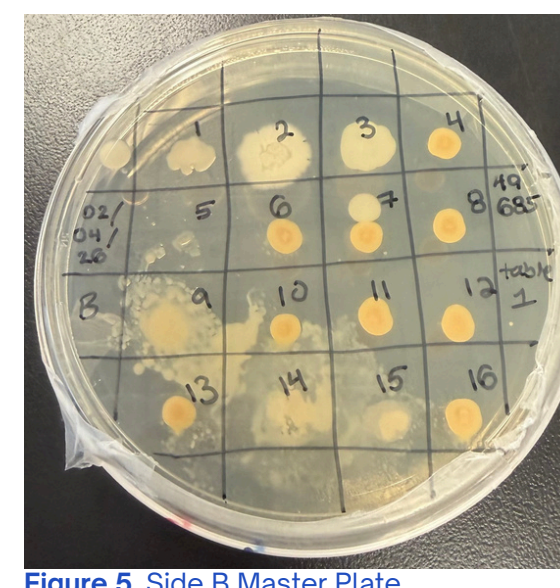


Figure 5. Side B Master Plate

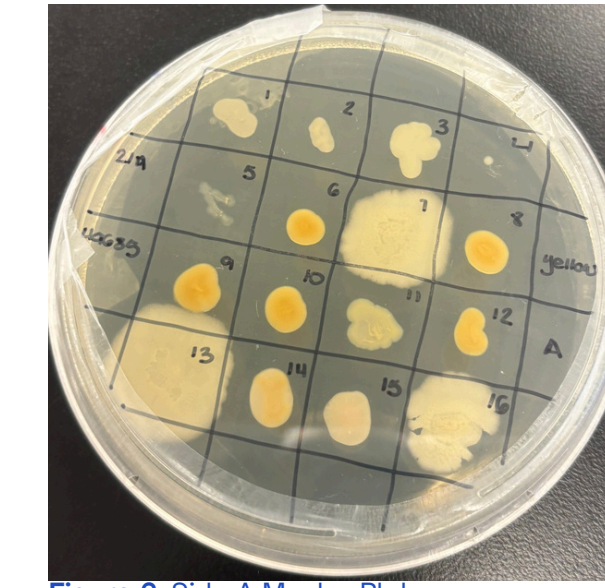


Figure 6. Side A Master Plate

Test for Gram Type, Antibiotic Production and Resistance

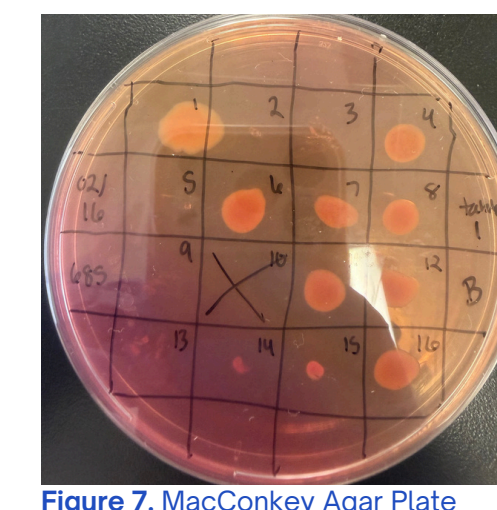


Figure 7. MacConkey Agar Plate

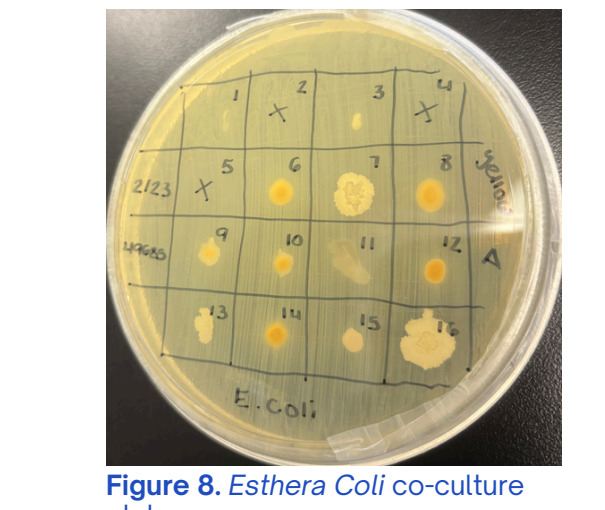


Figure 8. Escheria coli co-culture plate

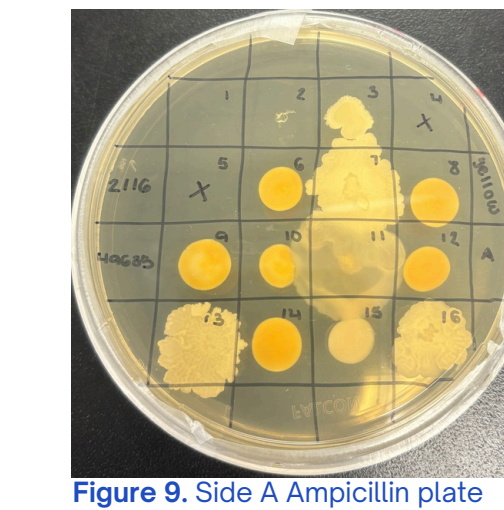


Figure 9. Side A Ampicillin plate

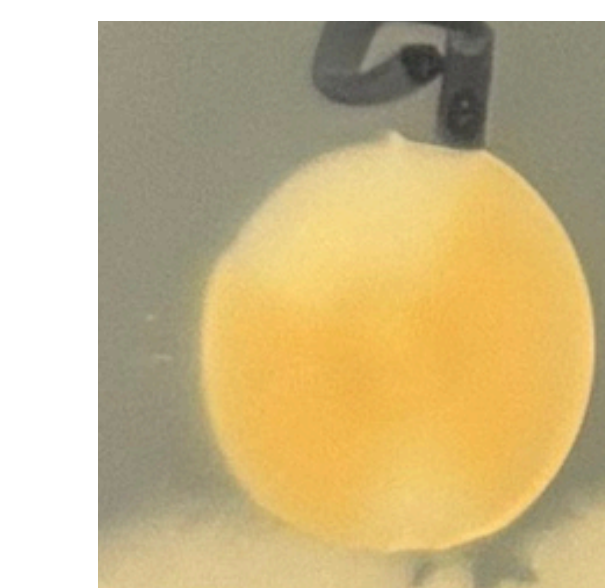


Figure 21. Colony was selected to be on a Master Plate because of its marble coloring, later found was two colonies growing together and chose to further study the white colony

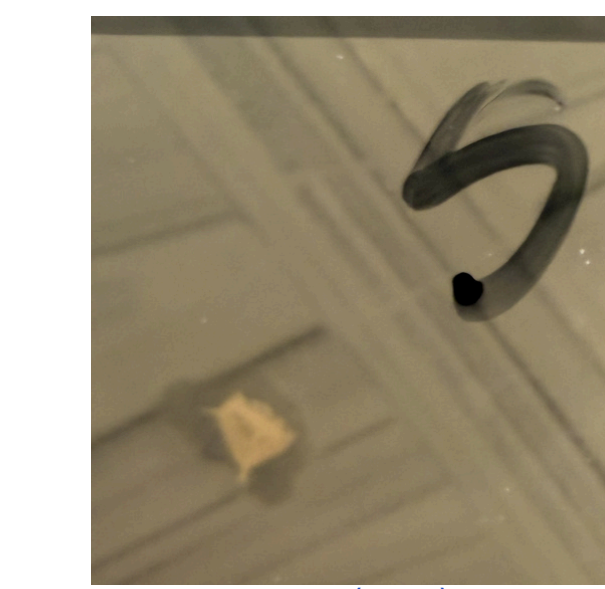


Figure 22. Colony 5 (side B) had a ZOI against Escherichia coli

Isolation of Pure Cultures via Streaking

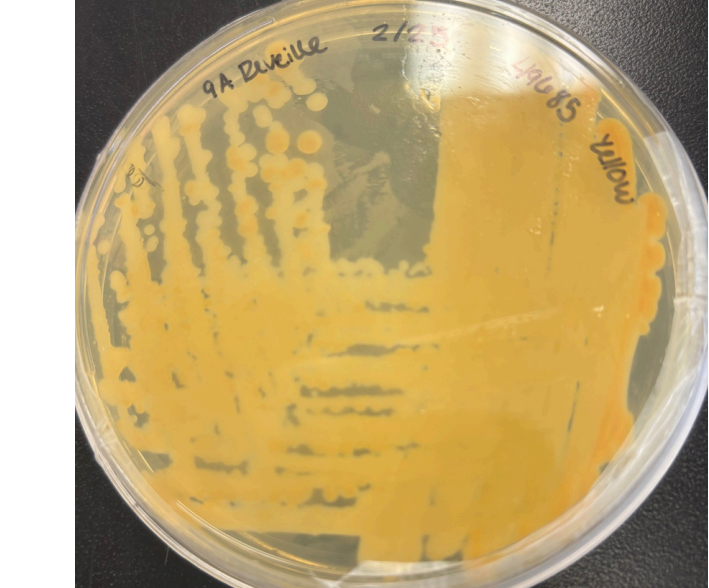


Figure 10. Reveille streak plate with both colonies



Figure 11. Oiled plate containing the 4 isolates

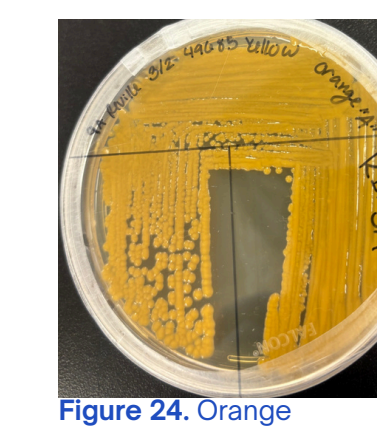


Figure 24. Orange pigmented Reveille colony



Figure 23. White pigmented Reveille colony

16S rRNA PCR and Gel Electrophoresis

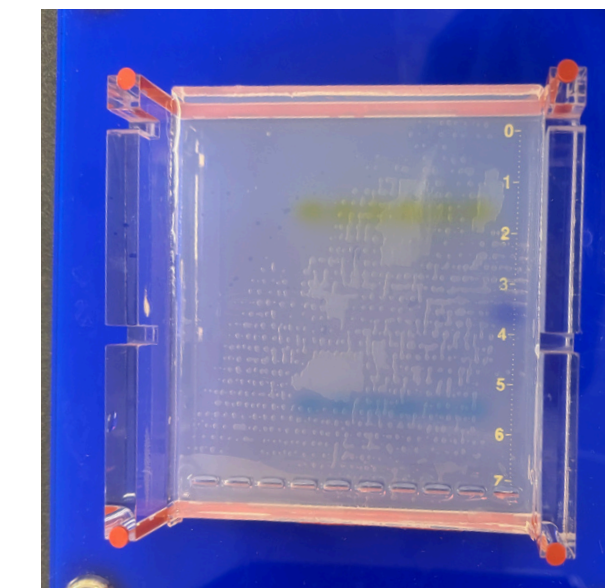


Figure 12. Gel before LEE, blue indicates start and yellow indicates end

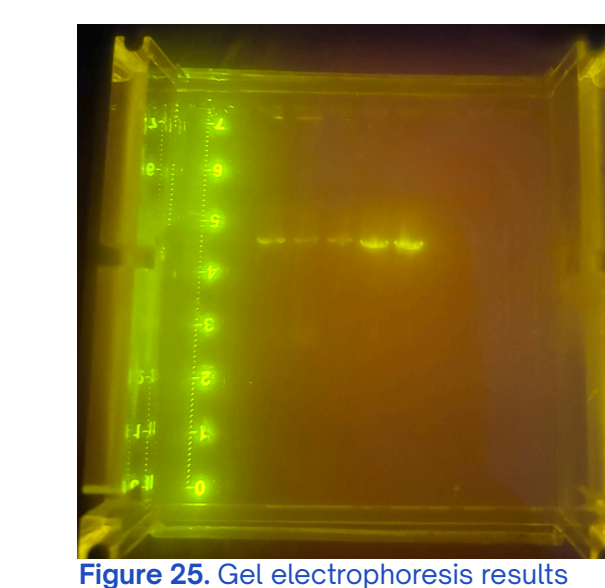


Figure 25. Gel electrophoresis results showed bands for all 4 isolates on the gel

Microscopy and Gram Staining

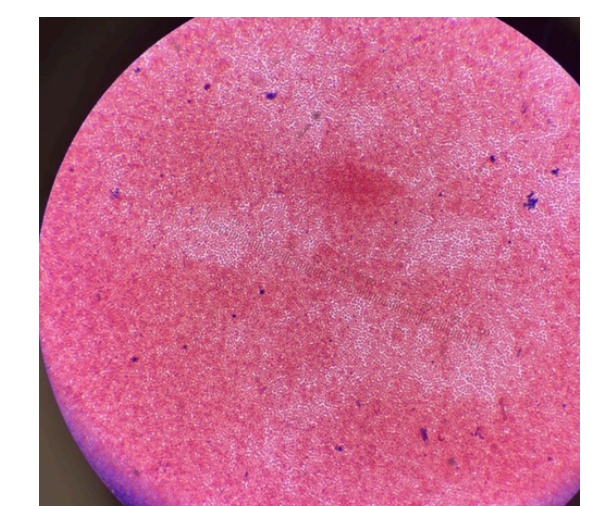


Figure 13. Bella gram-stain microscopy results (gram-negative)

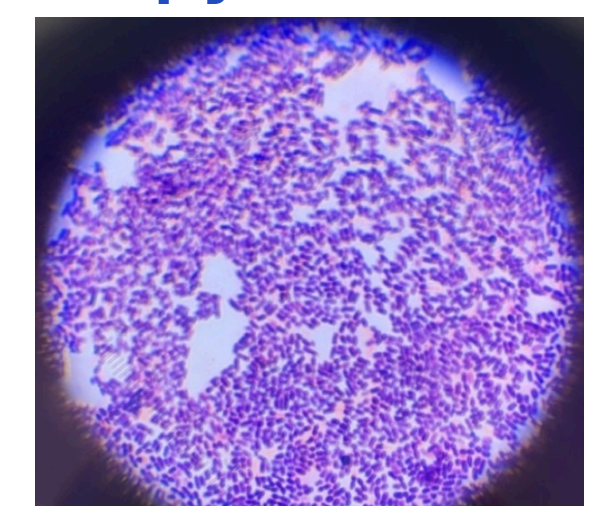


Figure 14. Charlie gram-stain microscopy results (gram-positive)

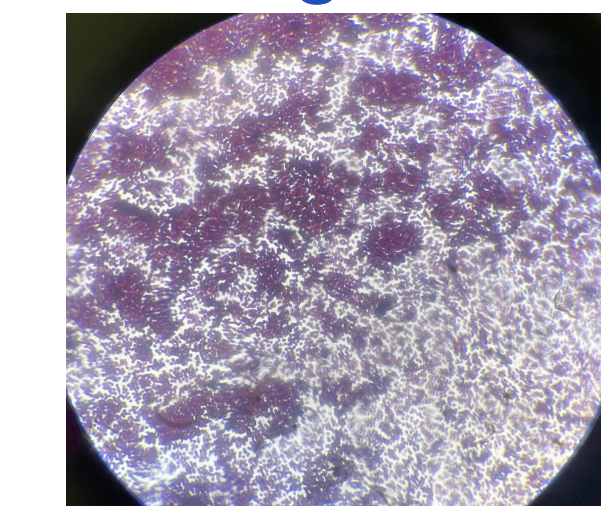


Figure 15. Reveille gram-stain microscopy results (gram-positive)

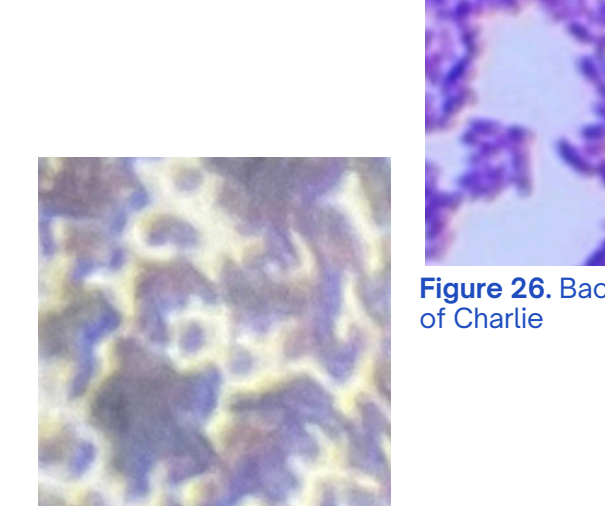


Figure 26. Bacillus cells of Charlie

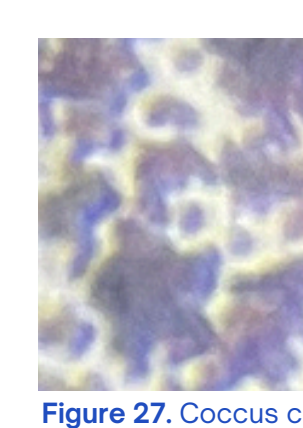


Figure 27. Coccus cells of Reveille

BLAST

Name	Max Score	Query Coverage	E Value	Percent Identity	Description
Chryseobacterium Indologenes	2012	96%	0.0	97.16%	Gram negative, rod shaped, ampicillin resistant, chloramphenicol resistant
Chryseobacterium Gleum	2001	96%	0.0	96.91%	Gram negative, rod shaped, ampicillin resistant, chloramphenicol resistant
Chryseobacterium Anthroshaene	2001	96%	0.0	96.99%	Gram negative, rod shaped, ampicillin resistant, generally chloramphenicol resistant

Figure 16. Use gram-stain microscopy results (gram-negative)

Figure 17. BLAST bacteria matches to Dia

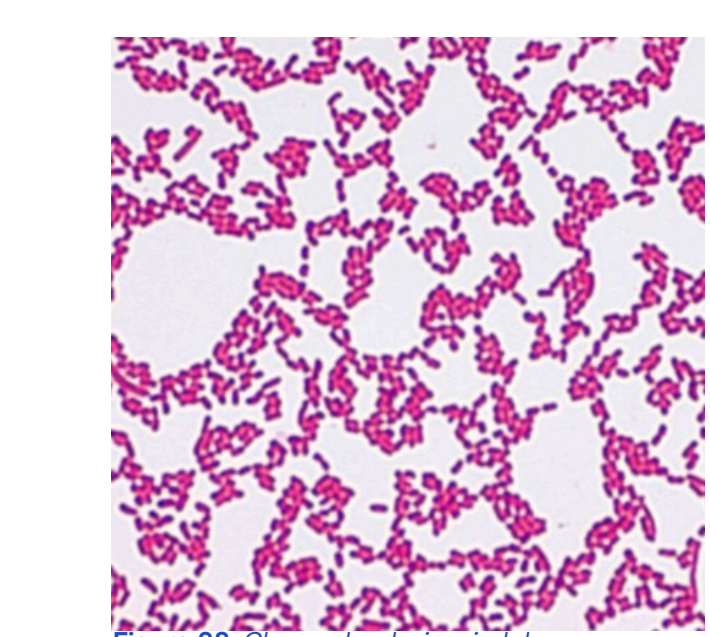


Figure 28. Chryseobacterium indologenes gram stain (Microbe Canvas, n.d.)

Results

The sample was collected after a freeze in a Savannah habitat, 10 feet away from trees. The dominant flora nearby were: *Juniperus ashei*, *Ulmus crassifolia*, *Quercus fusiformis*, and *Opuntia engelmannii* var. *lindheimeri*.

Each of the five different dilution samples was spread onto five labeled Tryptic Soy Agar (TSA) plates. Then, 32 colonies were chosen from 10⁻¹ and 10⁻² dilution plates. Each Master Plate contained 16 colonies, and the group observed 1,840,000 CFU's per gram of soil.

Colonies #1 and #11 (side B) had a Zone of Inhibition. The majority of the colonies from the entire group had a morphology of colored (25%), flat (43.75%), smooth (75%), opaque (68.75%), and mucoid (50%).

65.5% of the colonies were Gram-negative. Colonies #14 and #15 (side B) and #12 (side A) were lactose-fermenting. 75.9% of the colonies were resistant to Ampicillin, 58.7% to Chloramphenicol, and 58.7% to both. Only colony #5 (side B) produced antibiotics against *Escherichia coli*. None produced antibiotics against *Enterococcus raffinosus*.

Four colonies from the two main Master Plates were isolated to examine further. The isolates were side A colony #9, side A colony #12, side B colony #1, and side B colony #5. The names were Bella (1B), Charlie (5B), Reveille (9A), and Dia (12A). Each isolate was streaked onto an individual plate. A quad plate was created with all four isolates on it. Here we discovered Reveille was actually two colonies by streaking white and orange parts separately.

The four isolates underwent PCR to amplify the 16s rRNA gene within each isolate. The PCR products were run on an agar gel using electrophoresis. Each isolate produced bands on the gel which confirmed that the isolates were bacterial.

The isolates were stained to determine Gram type and examined under a microscope to determine cell morphology. Dia was Gram-negative & bacillus. Reveille (white colony) was Gram-positive & coccus. Bella was Gram-negative & coccus. Charlie was Gram-positive & bacillus.

Isolate Dia was sent for sequencing analysis. The DNA was sequenced using Sanger sequencing. This is a low throughput technique & sequences one fragment at a time. The result is the nucleotide order of 16s rRNA. Our isolate had a 97.16% match to *Chryseobacterium Indologenes*. It is found in soil, plants, and water sources. It is resistant to Beta Lactam antibiotics which includes ampicillin.

Our Colony Data vs Class Data

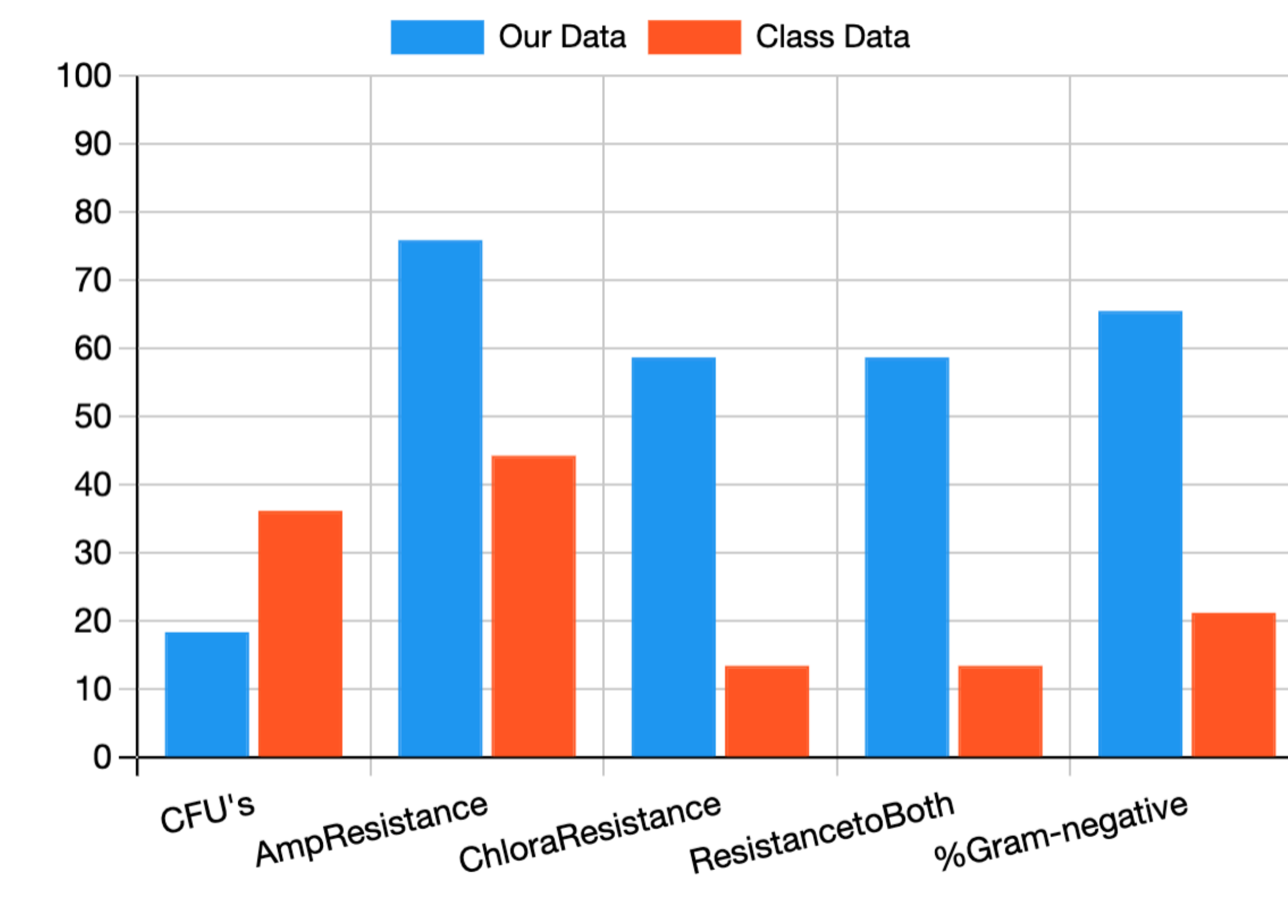


Figure 29. Graph showing a comparison of our group's data (blue) and the average class data (red). It compared Colony Forming Units, Ampicillin Resistance, Chloramphenicol Resistance, Resistance to both Antibiotics, and the percentage of colonies that were Gram-negative.

Conclusion

Our study of the Savannah habitat at the Lady Bird Johnson Wildflower Center revealed a relatively low microbial density of 1,840,000 CFU per gram of soil. Instead of poor viability, this likely reflects a highly competitive soil environment shaped by the root systems of native *Juniperus ashei* and *Opuntia engelmannii*. These plants, particularly *Juniperus ashei*, are known to release antimicrobial compounds that inhibit some microbes, especially Gram-positive bacteria. This selective environment is reflected in both the microbial composition and resistance patterns observed in our sample. Out of the 29 isolated colonies, the vast majority were Gram-negative, which contrasts with typical savannah soils that often contain more Gram-positive organisms. The dominance of Gram-negative bacteria aligns with the high levels of antibiotic resistance observed, with 75.9% of isolates resistant to Ampicillin, 58.6% resistant to Chloramphenicol, and 58.69% resistant to both. BLAST sequencing results further support this interpretation. Isolate Dia showed a 97.16% match to *Chryseobacterium indologenes*, a Gram-negative, rod-shaped (bacillus) bacterium commonly found in soil, plant-associated environments, and water sources. The Gram-negative classification is also consistent with the observed resistance trends, as these bacteria are more resistant to many antibiotics due to structural features. Even within this highly competitive and resistant environment, only colony Charlie successfully produced antibiotics against *Escherichia coli*, while none inhibited *Enterococcus raffinosus*. The identification of *Chryseobacterium indologenes* alongside this resistance-dominated community strengthens the conclusion that the soil beneath *Juniperus ashei* and *Opuntia engelmannii* supports specialized, highly adapted microbes. The isolation of colony Charlie demonstrates that this location is a promising environment for novel antibiotic discovery. Some limitations are that that antibiotic screening was limited to *Escherichia coli* and *Enterococcus raffinosus*, meaning potential inhibitory effects against other pathogens remain unknown. Additionally, Sanger sequencing limits our genetic understanding of the community to a single isolate (Dia). Future research should focus on identifying the specific antibiotic compound produced by Charlie, investigating the genetic mechanisms underlying resistance in isolates such as *Chryseobacterium indologenes*, and expanding sequencing efforts to further characterize microbial diversity within this plant-influenced soil microbiome.

References

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- Spengler, György, et al. "Evaluation of the Antimicrobial and Antiviral Potential of Essential Oils against Antibiotic and Antifungal Resistance." *Molecules*, 2022.